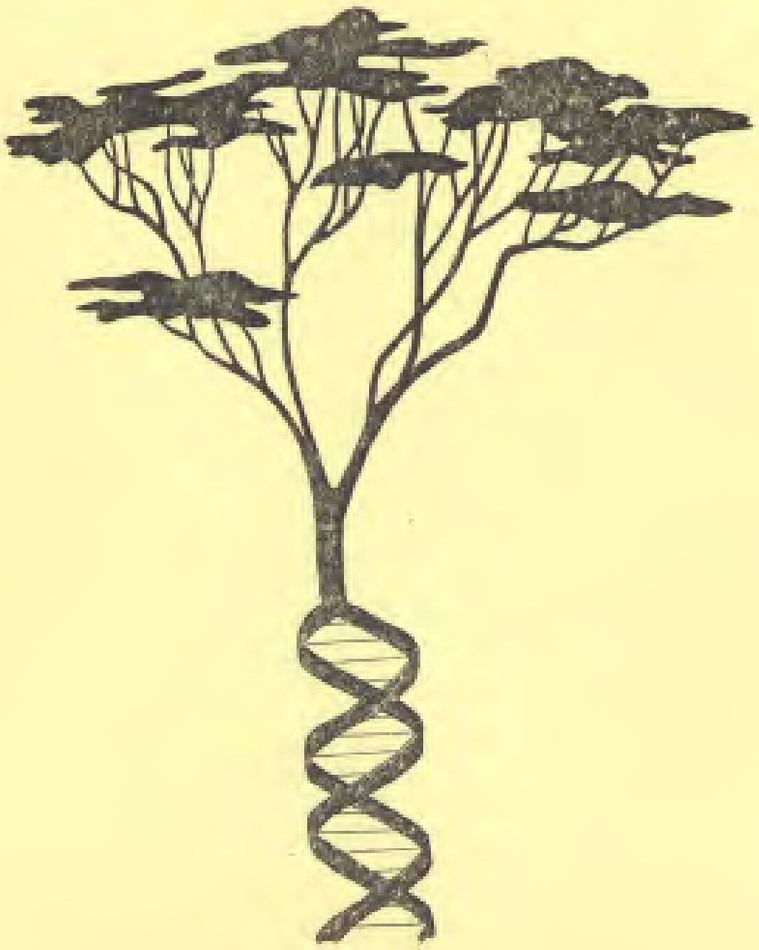
Fifteenth Annual Meeting

Mid-Atlantic Plant Molecular Biology Society



July 20-21, 1998
Laurel, MD

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INTRODUCTION

Welcome to the fifteenth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. These meetings are designed to bring some of the best scientific minds to our area and to introduce some of the most interesting advances in plant molecular biology to our area scientists at a reasonable price and at an accessible location. We hope to entice a large number of students, postdocs and senior scientists to attend and actively participate in presentations and discussions. In addition, the meeting is designed to encourage mixing of scientists in an informal atmosphere during on-site lunches and breaks to provide each participant the opportunity to meet invited speakers and other members.

The meeting encompasses a large number of important research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests. Please contact members of the organizing committees if you have thoughts or comments for consideration in the planning of future meetings; or join next years organizing team and volunteer your services to improve upon what we did this year. All are welcome at every stage of planning and organizing each meeting.

Many people were involved in the organization and planning of the meeting, and we give them out hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date product advances, and help to defray costs. Please visit our sponsors' and exhibitors' displays which are located with the posters. The level of interest you show in their products is a critical factor in their willingness to support future meetings.

We thank you for your continued support of and participation in the MidAtlantic Plant Molecular Biology Society. Enjoy the meeting!

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1998 MAPMBS MEETING SCHEDULE

Monday, July 20

8:30 a.m. Registration and Poster Set-up

9:10 a.m. Opening Remarks - Ben Matthews

Plant-Microbe Interactions
(Moderator: John Hammond, USDA-ARS)

9:15 a.m. Valerian Dolja (Oregon State University, Corvallis, OR). Large RNA genome of a closterovirus as a tool of plant molecular biology, virology, and biotechnology.

9:45 a.m. Rosemarie Hammond (USDA, Beltsville, MD). Exploitation of plant viral vectors for the study of viroid:host interactions.

10:15 a.m. Reid Frederick, Roger Thilmony, Guido Sessa, Gregory Martin (USDA, Frederick, MD and Purdue University, West Lafayette, IN). Recognition-specificity for AVRPTO is determined by threonine-204 in PTO.

10:35 a.m. COFFEE BREAK/POSTERS (Set-up and Viewing)/EXHIBITORS

Plant-Microbe Interactions (continued)

11:05 a.m. Terrence Delaney (Cornell University, Ithaca, NY). Molecular genetic studies of systemic acquired resistance and other induced forms of disease resistance.

11:35 a.m. Ralph Scorza, Laurene Levy, Vern Damsteegt, Ann Callahan, Kevin Webb (USDA, Kearneysville, WV; USDA, Beltsville, MD; USDA, Frederick, MD; INRA, France). Coat protein-mediated resistance to plum pox virus in *Prunus domestica* and transfer of resistance through hybridization.

11:55 a.m. LUNCH/EXHIBITORS/POSTERS

Genomes and Evolution
(Moderator: David Smith, USDA-ARS)

1:15 p.m. Joachim Messing, Victor Llaca (Rutgers University, Piscataway, NJ). Comparative genome analysis of a gene-dense region of *Zea mays* with regions of *Sorghum bicolor* and *Oryza sativa*.

1:45 p.m. Jonathan Wendel (Iowa State University, Ames, IA). Jeans, genes, and the origin of species.

2:15 p.m. Brian D. Esau, V. Frankard, M. Jacobs, B.F. Matthews (USDA, Beltsville, MD and Vrije Universiteit Brussel, Belgium). Isolation and characterization of a monofunctional aspartokinase cDNA from soybean.

2:35 p.m. Gary R. Kinard, M.A. Guaragna, R.L. Jordan (USDA, Beltsville, MD). Genome organization of pelargonium line pattern virus.

2:55 p.m. Business meeting

3:05 p.m. COFFEE BREAK/EXHIBITORS/POSTERS

KEYNOTE ADDRESS
(Introduction: Jonathon Arias, University of Maryland)

3:45 p.m. Roger Beachey (The Scripps Research Institute, La Jolla, CA). Rational design of mutants of the coat and movement proteins of tobacco mosaic virus to effect pathogen-derived resistance.

Tuesday, July 21

Gene Regulation and Plant Development
(Moderator: Margaret Pooler, USDA-ARS)

9:00 a.m. Mauricio Bustos (University of Maryland Baltimore County, Baltimore, MD). Gene transcription plays a central role in the regulation of seed maturation and dormancy.

9:30 a.m. Paul Larsen, Jason Shockey, Caren Chang (University of Maryland, College Park, MD). Exploring the interaction between ETR1 and CTR1: Its role in etyhlene signal transduction in Arabidopsis.

9:50 a.m. David Smith, Kenneth Gross (USDA, Beltsville, MD). Role of Beta-galactosidase in tomato fruit ripening and development.

10:10 a.m. A. Bruce Cahoon, Michael Timko (University of Virginia, Charlottesville, VA). Characterization of nuclear genes required for light-independent chlorophyll synthesis in *C. reinhardtii.*

10:30 a.m. COFFEE BREAK/EXHIBITORS/POSTERS

Transgenic plants
(Moderator: Javier Castillon, USDA-ARS)

11:00 a.m. Rob Griesbach (USDA, Beltsville, MD). The effect of a pH gene on the color of Petunia flowers.

11:20 a.m. Alexander Vainstein, Amir Zuker, Asaph Ahroni, Tzvi Tzfira, Marianna Ovadis, Hanan Itzhaki, Elena Shklarman, Hagit Ben-Meir (The Hebrew University of Jerusalem, Rehovot, Israel). Application of an integrative system based on microprojectile bombardment and Agrobacterium tumefaciens to generate transgenic carnation plants with novel characteristics.

11:40 a.m. Jhy-Jhu Lin (Life Technologies, Inc., Rockville, MD). Interactions between plant auxin and antibiotics and the stability of plant auxin on plant tranformation.

12:00 am LUNCH/EXHIBITORS/POSTERS

Parasitism, pest resistance, and thermotolerance
(Moderator: Frank Turano, USDA-ARS)

1:15 p.m. David Lynn (University of Chicago, Chicago, IL). Evolution of parasitism in plants: the best offense is a leaky defense.

1:45 p.m. Benjamin F. Matthews, Margaret MacDonald, Ruth Heinz (USDA, Beltsville, MD). Differentially expressed genes induced in resistant soybean plants in response to soybean cyst nematode, *Heterodera glycines*, invasion.

2:05 p.m. Janet Slovin, Malik Mukesh, Lynn Zimmerman (USDA, Beltsville, MD and University of Maryland, Baltimore County, MD). Transformation of carrot and tomato with a carrot heat shock protein gene, HSP17.7, driven by the CaMV 35S promoter, enhances thermotolerance in cell lines and/or plants.

2:25 p.m. Closing Remarks - Ben Matthews

2:30 p.m TAKE DOWN POSTERS

1998 MAPMBS POSTER SESSIONS

9	38	Jennifer Hansen, Lori Lasco, Jessica Chinault, John Kerrigan, Mary Fields, Robert Dawley (Ursinus College, Collegeville, PA). The genomes of rapid-cycling brassicas are no smaller than those of their wild-type progenitors.
10	39	R. Todd Leister and Fumiaki Katagiri (Univeristy of Maryland Baltimore County, Baltimore, MD). Transient expression of *RPM1* and *RPS2* with their cognate avirulence genes in *Arabidopsis* protoplasts to study gene-for-gene disease resistance.
11	40	Michelle Wood, Michael Lidell, Steven Hutcheson, Fumiaki Katagiri (University of Maryland Baltimore County, Baltimore, MD and University of Maryland, College Park, MD). Novel approach to detect protein transfer from a bacterial pathogen to its plant host.
12	41	N. Assad-Garcia, Jhy-Jhu Lin, Jiu-Lin Xia, Jianquing Lan (Life Technologies, Inc., Rockville, MD). The effect of tissue culture conditions on auxin stability in culture media.
13	42	Jonathan Kuo, Jhy-Jhu Lin (Life Technologies, Inc., Rockville, MD). Plant nucleic acid isolation using guanidine based reagents.
14	43	Jhy-Jhu Lin, Lee Sheng, Ryan Fleming,k Nacyra Assad-Garcia (Life Technologies, Inc., Rockville, MD). Comparing digital images to conventional photographs.

LARGE RNA GENOME OF A CLOSTEROVIRUS AS A TOOL OF PLANT MOLECULAR BIOLOGY, VIROLOGY, AND BIOTECHNOLOGY

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Department of Botany and Plant Pathology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR 97331

The closteroviruses possess filamentous particles encapsidating 15 to 20 kb RNA genomes that are the largest among all known viruses of plants. Plant-to-plant transmission of closteroviruses is mediated by insects acquiring virions during the feeding on phloem sieve elements. Although closteroviruses exhibit strong phloem tropism, they are capable of unloading from the phloem into leaf mesophyll cells. We have generated the 15.5 kb cDNA clone of the beet yellows closterovirus (BYV) from which infectious RNA transcripts can be derived. This clone has been used to map the functions encoded in BYV genome and to test its utility as a gene expression vector. It was revealed that the multidomain proteins encoded in BYV ORFs 1a and 1b are essential for RNA replication. Two additional proteins, a leader proteinase (L-Pro) and a 21-kDa protein (p21) were required for efficient amplification of the genome. In contrast, the genes coding for a homolog of cellular HSP70 molecular chaperones, two capsid proteins (CPs), and three nonstructural proteins were superfluous for RNA amplification. It was also found that the L-Pro is required for viral infectivity in whole plants, but not in the isolated protoplasts suggesting that L-Pro is involved in a shut-off of a plant defense response. The cDNA clone was further modified via insertion of a reporter ORF between first and second codons of the CP ORF. This reporter ORF encoded a fusion protein composed of beta-glucuronidase (GUS) and proteinase domain derived from potyvirus. The expression of hybrid ORF driven by CP promoter resulted in accumulation of enzymatically-active GUS-Pro and free CP released due to autoproteolytic activity of the Pro domain. This novel strategy of tagging allows expression of a reporter and of a virutually unchanged gene product from any viral or plant promoter. The ability of BYV to produce ~90-kDa reporter protein highlights a potential of closteroviruses as high-capacity, phloem-specific gene expression vectors.

EXPLOITATION OF PLANT VIRAL VECTORS FOR THE STUDY OF VIROID-HOST INTERACTIONS

ROSEMARIE HAMMOND, Yan Zhao, and Robert A. Owens.
USDA/ARS Molecular Plant Pathology Laboratory, Beltsville, MD 20705

Viroids are the smallest known phytopathogens and infect many economically important crop plants. Potato spindle tuber viroid (PSTVd) is a covalently closed circular RNA molecule of 359 nucleotides that replicates autonomously, presumably within the nucleus of host cells. We are exploiting plant viral-based vectors to transiently express sequences to facilitate the study of various aspects of viroid/host interactions, including movement and pathogenesis.

To approach the identification of specific nuclear targeting sequence elements residing in the viroid molecule, we have developed an *in vivo* reporter system using green fluorescent protein (GFP) as the reporter molecule. The coding region of GFP was interrupted by insertion of an intron derived from IV2 of the potato ST-LS-1 gene. A complete PSTVd sequence was embedded within the intron, and this construct was delivered into *Nicotiana benthamiana* via a potato virus X (PVX)-based vector. The intron-containing GFP reporter gene expressed as a subgenomic RNA in the cytoplasm will not produce a functional GFP unless the subgenomic RNA is targeted to the nucleus, where the intron can be removed and the spliced RNA returned to the cytoplasm and translated. The appearance of green fluorescence in mesophyll, epidermal, and vesicular tissues of leaves of plants inoculated with constructs containing a full-length PSTVd molecule embedded within the intron indicates that PSTVd sequences facilitated nuclear targeting and that RNA splicing events have occurred. Direct evidence that a splicing event occurred was obtained by RT-PCR analysis of nucleic acids extracted from leaves two weeks post-inoculation.

In many cases, viroid infection results in symptoms of stunting, epinasty,and vein clearing. We are studying the molecular basis of the response of 'Rutgers' tomato to infection by potato spindle tuber viroid (PSTVd). Protein phosphorylation may play a role in this response. Expression of a specific protein kinase, hereafter termed PKV, is transcriptionally activated in plants infected with PSTVd strains causing intermediate and severe symptoms and is expressed at low levels in mock inoculated or plants inoculated with the mild strain of PSTVd. A full-length genomic DNA clone of the protein kinase gene has been isolated and sequence analysis shows that it has significant homologies to cyclic nucleotide-dependent protein kinases. We are examining the molecular interactions between viroid molecules and the protein kinase and are using viral-based vectors to deliver sense and antisense copies of the gene to tomato to examine the biological role of PKV in viroid symptom induction.

RECOGNITION-SPECIFICITY FOR AVRPTO IS DETERMINED BY THREONINE-204 IN PTO.

Reid Frederick[1], Roger Thilmony[2], Guido Sessa[2] and Gregory Martin[2], [1]USDA-ARS-NAA, Foreign Disease-Weed Science Research Unit, Fort Detrick, Frederick, MD 21702, [2]Dept. of Agronomy, Purdue University, West Lafayette, IN 47907

Plant disease resistance is often initiated by a recognition event specified by a host resistance gene and a corresponding pathogen avirulence gene. Recognition of the invading pathogen by the host triggers a hypersensitive response (HR) typified by rapid, localized death of host cells. In tomato, *Pto* encodes a serine/threonine kinase which confers resistance to *Pseudomas syringae* pv. *tomato* strains expressing the *avrPto* gene. In previous work with the yeast two-hybrid system, it was shown that Pto physically interacts with AvrPto and this recognition is very specific; AvrPto does not interact with the Fen kinase which shares 80% amino acid identity with Pto. Using Pto-Fen chimeric proteins and site-directed mutagenesis we found that threonine-204 located in subdomain VIII is required for Pto interaction with AvrPto in a yeast two-hybrid system and also for recognition-specificity in a tobacco leaf transient assay. Substitution of this residue in the Fen kinase allowed it to interact with AvrPto and to confer an *avrPto*-specific HR in tobacco leaves.

COAT PROTEIN-MEDIATED RESISTANCE TO PLUM POX VIRUS IN *PRUNUS DOMESTICA* AND TRANSFER OF RESISTANCE THROUGH HYBRIDIZATION.

Ralph Scorza[1], Laurene Levy[2], Vern Damsteegt[3], Ann Callahan[1], Kevin Webb[1], Michel Ravelonandro[4].* [1]USDA-ARS Appalachian Fruit Research Station, 45 Wiltshire Rd., Kearneysville, WV 24530; [2]USDA-APHIS, PPQ, Plant Methods Development Laboratory, Bldg 580, Beltsville, MD 20705; [3]USDA-ARS Foreign Disease-Weed Science Research Unit, Fort Detrick, Frederick, MD 21702; [4] Station de Pathologie Vegetal, INRA, Centre de Recherches de Bordeaux, BP 81 33883 Villenave d'Ornon, France.

Virus diseases of stone fruits are of major concern. They can cause yield reductions, crop losses and death of trees. They are a major impediment to the worldwide exchange of *Prunus* germplasm. Control is usually indirect through the use of insecticides and nematicides to control vectors. Certification programs are also used to control the spread of disease. There are few examples of virus resistance in *Prunus*, thus genetic engineering represents a potentially useful approach to obtain resistant germplasm. We are using plum pox virus (PPV) as a model for developing virus resistance *Prunus* through genetic engineering. Transgenic plums containing the PPV coat protein (CP) or the related papaya ringspot virus (PRV)-CP gene were produced through *Agrobacterium tumefaciens*-mediated transformation. These transgenic plum clones were then evaluated for resistance to PPV infection in the greenhouse by graft or aphid inoculation with PPV. While symptoms of PPV appeared in most transgenic clones, all plants of PPV-CP transgenic clone C5 were symptomless and ELISA and immunocapture-reverse transcriptase PCR negative for over three years following inoculation with two strains of PPV (Ravelonandro et al., Plant Dis 81:1231-1235, 1997). Transgenic clone C5 contains mulitple copies of the PPV-CP insert. It produces low levels of PPV-CP RNA and undetectable levels of CP. The mechanism of resistance appears to be one of co-suppression. Pollen of clone C5 was applied to flowers of PRV-CP transgenic plants. Progeny were obtained containing no transgenes, only the PPV-CP, only the PRV-CP, or both the PRV-CP and PPV-CP transgenes. The multicopy inserts of either the PPV-CP or the PRV-CP transgenes appeared to be inherited as closely linked blocks of genes resembling single gene inheritance. Seedlings were inoculated with PPV. At 5 and 11 months post-inoculation, seedlings containing the PPV-CP genes from C5 were symptomless and ELISA negative. Seedlings containing only PRV-CP transgenes or non-transformed controls showed symptoms of PPV infection and were ELISA positive. These results indicate that the PPV-CP transgenes can be transferred to progeny through hybridization and that these genes can impart resistance to PPV in transgenic seedlings. The mechanism of resistance in the progeny, the combined effects of both transgenes on resistance to PPV, and the stability of PPV resistance in the progeny of the resistant C5 transgenic line are currently under evaluation.

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY SYMPOSIUM

July 20-21, 1998

COMPARATIVE GENOME ANALYSIS OF A GENE-DENSE REGION OF *Zea mays* WITH REGIONS OF *Sorghum bicolor* AND *Oryza sativa*.

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Abstract

Zeins are the major storage proteins present in maize endosperm. The most abundant class, alpha-zeins, includes 19- and 22-kDa proteins encoded by a multigene family. In the inbred line BSSS53, most if not all 22-kDa alpha-zein gene related sequences are clustered in one location on the short arm of chromosome 4. The post-transcriptional regulator *dzr1*, responsible for the overexpression of a methionine-rich delta zein in BSSS53, also maps to this location. To characterize the organization of these genes in this region, we have constructed a high-resolution genetic and physical map. We have shown by long-range restriction mapping that the 3.4-cM 22-kDa alpha-zein cluster region spans less than 250 kb, and includes two subclusters of zein genes, separated by a large (70kb) intervening spacer region. However, when an attempt was made to form a contiguous set of overlapping cosmids, two gaps remained due to the retroelements present in the intervening spacer region. Therefore, we have taken advantage of the synteny between maize and sorghum to establish the physical linkage between a zein subcluster and the intervening spacer region. One sorghum BAC clone has been identified with an anchor probe from the central intervening spacer region and the 22-kDa zein genes, allowing a micro-syntenic alignment between chromosome 4S of maize and sorghum complementation group G. Several BAC clones from *Oryza sativa* L. ssp. *indica*, cv Teqing and from ssp. *japonica*, cv Nipponbare have been isolated using the central anchor probe. Sorghum and rice BAC libraries have been provided by Dr. Rod Wing, Clemson University, SC. Although alpha zein genes are present in the sorghum genome, it is already known that they have formed recently in evolutionary terms and are absent in rice. Therefore, we would expect zein genes to represent an insertion relative to the rice genome. First, a 78.1 kb sequence from maize has been sequenced representing one of the subclusters. This subcluster contains ten tandemly arranged zein-related sequences, three other additional predicted genes on the same DNA strand, one on the other DNA strand, and extensive evidence of transposition events. One of the non-zein gene sequences shows homology to a rice EST. With gene sequences spaced nearly every 5 kb, this region resembles more the gene density found in *Arabidopsis thaliana* than the larger cereal genomes. However, it appears that seven of the zein genes do not have a full length open reading frame caused by mutations in the glutamine codon CAG or CAA that result in TAG and TAA stop codons. Interestingly, one orthologous gene in another inbred line, W22, has one in-frame stop codon, where as the gene in BSSS53 has none and is expressed at normal levels. Therefore, we propose two gene pools one that is a reserve for the other and where weakly expressed genes could switch to normally expressed ones by gene conversion of orthologous sequences of less than one kilobase.

ISOLATION AND CHARACTERIZATION OF A MONOFUNCTIONAL ASPARTOKINASE cDNA FROM SOYBEAN

Esau BD[1], Frankard V[2], Jacobs M[2] and Matthews BF[1]
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We have amplified a PCR fragment from a cDNA library prepared from PolyA RNA isolated from *Glycine max* cv. Century grown for 6 days in the dark. This fragment has homology to two *Arabidopsis thaliana* monofunctional aspartokinase clones. The amino acid translation of this soybean clone has approximately 80% identity to the amino acid sequence of either of the *Arabidopsis* clones. Reverse transcription of *G max* cv. Century PolyA RNA yielded a PCR fragment coding for an 88 amino acid transit peptide. Comparison of the amino acid sequence of this aspartokinase to other monofunctional and bifunctional aspartokinases showed a higher degree of homology to the *E coli* monofunctional isoform than to the bifunctional isoform from soybean suggesting that the monofunctional and bifunctional isoforms constitute two distinct lines separating early in evolution. This soybean monofunctional clone contains the same three highly conserved amino acid motifs present in all aspartokinases sequenced so far. However, there are slight differences in these motifs between the monofuntional and bifunctional isoforms. A northern blot of PolyA RNA isolated from soybean cotyledons and leaves grown in the dark and light for 4 or 8 days was hybridized with the soybean aspartokinase clone. In all cases a single band of 2.25 kb was obtained. The level of transcript appeared to be higher in tissues of light grown seedlings in all cases.

GENOME ORGANIZATION OF PELARGONIUM LINE PATTERN VIRUS

G.R. Kinard, M.A. Guaragna, and R.L. Jordan. USDA-ARS, US National Arboretum, Floral and Nursery Plants Research Unit, Beltsville, MD, 20705.

Pelargonium line pattern virus (PLPV) consists of monopartite single stranded RNA encapsidated as 30 nm isometric particles with a coat protein of about 37 K. The virus causes line and ring patterns on leaves of infected geraniums, which is the only known natural host for the virus. We are currently determining the complete nucleotide sequence of PLPV using a strategy of overlapping cDNA clones and primer extensions with dye labelled terminators. The 3' terminus was verified by poly-A tail addition followed by cDNA synthesis with oligo T and the 5' terminus is being verified by cDNA synthesis with a genome-specific primer. The virus contains at least 3873 nt and is most closely related in sequence to members of the Carmovirus genus of the family Tombusviridae. Gene expression probably involves a read-through stop codon in the RNA dependent RNA polymerase and expression of the coat protein via subgenomic RNA. Northern blot assays using cloned DNA probes show that PLPV produces only one subgenomic RNA in infected plants. This suggests the gene expression strategy for PLPV is different than those of other carmoviruses, which produce two subgenomic RNA species. The putative p7/p9 movement proteins may be expressed as a fusion protein through a -1 ribosomal frameshift mechanism.

Rational Design of Mutants of the Coat and Movement Proteins of TMV to Increase Efficacy of Pathogen Derived Resistance

Roger N. Beachy, Mohammed Bendahmane, Bruce Dunsmore, Art Olson, Ted Kahn, Chae-Oh Lim, and Christoph Reichel

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During the past 15 years we developed transgenic plants that exhibit 'Pathogen Derived Resistance' to virus infections using the tobacco mosaic virus (TMV)-tobacco system and genes that encode the coat protein (CP) and defective (non-functional) mutants of the P30 movement protein (dMP) Earlier work led us to conclude that the CP interferes with an early stage in infection and prevents disassembly of TMV in plants and cells that contain CP. In contrast we propose that dMP interferes with disease by limiting local and systemic spread of infection.

We continue to characterize the structural and cellular basis of CP- and dMP-mediated resistance with the expectation that this will lead to developing transgenes with greater efficacy in agricultural biotechnology. In studies of CP-mediated resistance we recently demonstrated that certain mutants of CP, selected based upon the known 3-dimensional structure of the CP, confer greater levels of CP-mediated resistance than does wild type CP, while other mutants confer little or no resistance. High levels of resistance were correlated with the capacity of the CP to form stable aggregates of CP that took the form of virus like particles, or ordered paracrystalline arrays of CP. By using molecular modeling programs that predict protein-protein interactions, we developed a model that suggests that increased resistance is conferred by several different mutants because new hydrogen bonds are formed between the transgenic CP and CP on the challenge virus. This further decreases the likelihood that challenge virus will disassemble and cause an infection.

Unlike the CP, the 3-D structure of movement protein is not known, and only recently has information related to its subcellular localization been established; in some of these studies we fused MP with the green fluorescent protein (GFP) to identify sites at which MP is localized in infected tissues. From these studies we and others have speculated on the role of MP in cell-cell spread of TMV infection. We developed a series of mutants of the MP and determined the effect of the mutation on function of the MP and on the subcellular localization of the protein. Our current goals include identifying mutants of the MP that, when expressed in transgenic plants, restrict the functions of movement protein(s) produced during virus infection and thereby reduce the spread of virus within the plant.

References:

Kahn, T.W., M. Lapidot, M. Heinlein, C. Reichel, B. Cooper, R. Gafny, and R.N. Beachy. 1998. Domains of the TMV movement protein involved in subcellular localization. *Plant J.* (in press)

Bendahmane, M., J.H. Fitchen, G. Zhang, and R.N. Beachy. 1997. Studies of coat protein mediated resistance to TMV: Correlation between capacity of mutant coat protein assembly and CP-MR. J. Virol. 71:7942-7950.

Heinlein, M., H.S. Padgett, S. Gens, B. Pickard, S.J. Casper, B.L. Epel, and R.N. Beachy. 1998. Changing patterns of localization of TMV movement protein and replicase to endoplasmic reticulum and microtubules during infection.. *Plant Cell* (In press).

Gene transcription plays a central role in the regulation of seed maturation and dormancy. Mauricio M. Bustos, Craig Dubois, Maw-Shenq Chern, Andrew J. Bobb, Hans-Georg Eiben, and Izabella Balakirski. Department of Biological Sciences, University of Maryland, Baltimore County. 1000 Hilltop Circle, Baltimore, Maryland 21250.

Two main temporal programs of gene expression, MAT (early maturation) and LEA (late embryo abundant), occur after the cotyledon stage of embryogenesis in dicots. In Arabidopsis thaliana, a number of MAT- and LEA-type genes are affected by mutations in abi (ABA insensitive), lec (leafy cotyledon), and fus3 (fusca3) genes. So far, four of these regulatory genes have been cloned i.e. ABI3, ABI4, LEC1, and FUS3. In every case, the predicted protein products have homology to known families of transcription factors. ABI3 genes from Arabidopsis and Phaseolus encode acidic transcription activators orthologous to the VP1 protein of maize. In species of Arabidopsis, Phaseolus, and Brassica, ABI3 transactivation of MAT promoters has been linked to the presence of RY-repeats i.e. G/CCATGCnnG/C. In contrast, RY-repeats are not found on LEA promoters, suggesting the existence of different control mechanisms and accessory factors specific for each type of promoter. Also, several groups have obtained evidence for genetic interactions between ABI3 and other ABI genes, and LEC1 or FUS3. We used a yeast two-hybrid assay to isolate cDNAs encoding putative ABI3-interactive-proteins (AIPs). AIP4 contains an N-terminal "kelch" domain homologous to the human transcription factor "host cell factor (HCF)", and a C-terminal coiled-coil domain that binds to ABI3. In contrast, AIP6 contains a RING finger (Zn2+-binding) and a proline-rich domain. Using a PCR and Southern hybridization procedure, we have isolated Arabidopsis lines carrying T-DNA tagged mutant alleles of AIP4 and AIP6. The heterozygotes segregate progeny that exhibit normal morphology but reduced sensitivity to ABA, suggesting a possible involvement of AIP4 and AIP6 in ABA signal transduction. ABI3 activated transcription is also negatively regulated i.e. repressed, by two G-box binding bZIP proteins, ROM1 and ROM2, from Phaseolus vulgaris. Both ROM factors function as DNA binding site-dependent repressors. The N-terminal repression domain of ROM1 binds in yeast to the bHLH-LZ factor GIP1 from Arabidopsis. The identification of transcription factors that regulate maturation development represents a major step forward in our understanding of how MAT and LEA gene expression patterns are regulated. Future research will focus on mapping the genetic and physical interactions between ABI3, AIP4, AIP6, and other genes that regulate seed maturation and dormancy.

Exploring the Interaction between ETR1 and CTR1: Its Role in Ethylene Signal Transduction in Arabidopsis

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Ethylene is one of the five classic plant hormones, having profound effects on plant growth and development, including promotion of tissue senescence and fruit ripening. Genetic analysis using *Arabidopsis thaliana* has identified several components of the ethylene signal transduction pathway, including several putative ethylene receptors (e.g. ETR1 and ERS) that resemble bacterial "two-component" receptors. This approach has also identified CTR1, a negative regulator of this pathway that has several hallmarks of the Raf family of ser/thr kinases. Epistasis analysis indicates that CTR1 acts at or downstream of the ethylene receptors, yet the mechanism by which the receptors regulate CTR1 has yet to be elucidated. Work in our lab has recently shown, through the use of two-hybrid analysis in yeast, that both ETR1 and ERS directly interact with the amino-terminal domain of CTR1. This interaction is also observed using an *in vitro* protein association assay. These results provide further evidence that CTR1 acts downstream of ETR1 and ERS and suggest that the regulation of CTR1 activity involves a novel interaction with the ethylene receptors. Work to analyze these interactions *in planta* is currently underway through the introduction of a gene chimera, consisting of the amino-terminal portion of *CTR1* fused to the green fluorescent protein, into *Arabidopsis* mutants. Our work is supported by the USDA/NRICGP (95-37304-2218 and 97-35304-4921).

ROLE OF BETA-GALACTOSIDASE IN TOMATO FRUIT RIPENING AND DEVELOPMENT

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Since galactose is the most dynamic sugar residue in the cell wall of developing tomato fruit, we are attempting to determine the role of beta-galactosidase during tomato fruit ripening and its importance in the softening and/or ripening process. We have determined that a family of at least seven beta-galactosidase genes is expressed during fruit development, and six of the genes are expressed during ripening. The genes are expressed differentially during the development of tomato fruit and throughout various fruit tissues. Additional molecular characterization of the genes is underway to determine their expression profiles in the ripening mutants nor, rin and Nr as well as their response to ethylene. We have made antisense constructs, transformed tomato tissues and established R0 plants in the greenhouse. The R1 transgenic plants will be tested for suppressed beta-galactosidase gene expression and altered phenotypes.

Sequence analysis suggests that all the genes code for proteins containing a signal sequence. It has been predicted that most of the proteins are secreted. However, one is probably targeted to the chloroplast, and therefore may be involved in galactolipid degradation. A yeast protein expression system has been used to produce active beta-galactosidase from one cDNA. This beta-galactosidase can cleave galactosyl residues from galactosyl-containing substrates purified from tomato fruit cell wall. We are currently making yeast expression vectors to test the remaining cDNA-encoded enzymes for substrate specificities.

CHARACTERIZATION OF NUCLEAR GENES REQUIRED FOR LIGHT-INDEPENDENT CHLOROPHYLL SYNTHESIS IN *C. REINHARDTII*

A. Bruce Cahoon and Michael P. Timko

Protochlorophyllide (pchlide) reduction is a key step in chlorophyll (CHL) biosynthesis and chloroplast organogenesis. Two distinct mechanisms for pchlide reduction are present in the green alga *Chlamydomonas reinhardtii*. Like angiosperms, *C. reinhardtii* cells reduce pchlide to chlorophyllide (chlide) in a light-dependent manner catalyzed by the nuclear-encoded enzyme NADPH:protochlorophyllide oxidoreductase (POR). *C. reinhardtii* cells also synthesize significant amounts of CHL in the absence of light by a light-independent pchlide reduction reaction (LIPOR) that is present in photosynthetic organisms from bacteria to gymnosperms but missing in angiosperms. Although the catalytic mechanism and cofactor requirements for the LIPOR process are unknown, the products of three chloroplast genes (designated *chlL, chlN, and chlB*) and at least seven nuclear loci (designated *y*) have been demonstrated to be required for LIPOR activity. Mutations in either the plastid or nuclear genes result in the same "yellow-in-the-dark" phenotype, that is, in the absence of light CHL is not synthesized and there is a buildup of yellow pchlide. Presented will be evidence that chlL, chlN, and chlB are post-transcriptionally regulated in wild type cells with a greater abundance of chlL and chlN protein present in the dark than in the light. The abundance of the proteins appears to be regulated by light-derived energy. Also presented will be evidence that all but one of the y-mutants are adversely effected in their ability to accumulate the chlL protein subunit.

The effect of a pH gene on the color of *Petunia* flowers

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An in vivo system was developed to determine the effects of pH on naturally occurring, pigment complexes within cells. The in vivo system was based upon a controlling element inserted into the Ph6 gene. The controlling element mutation was crossed into a genetically marked *Petunia hybrida* line expressing known flavonoid pigments. Flowers expressing the controlling element were variegated with the mutant background lighter in color than the revertant sectors. In the progeny, several individuals were found that had sectors that were a different flower color than the background. In these flowers, the background had a higher pH than the sectors. An increase in pH less than 0.5 unit changed the color from red to blue. The data suggested that the Ph6 gene was a regulator gene which controlled both the pH and anthocyanin concentration.

APPLICATION OF AN INTEGRATIVE SYSTEM BASED ON MICROPROJECTILE BOMBARDMENT AND *AGROBACTERIUM TUMEFACIENS* TO GENERATE TRANSGENIC CARNATION PLANTS WITH NOVEL CHARACTERISTICS.

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The genetic engineering of carnation (*Dianthus caryophyllus* L.), which ranks third in the world flower market, is a highly desirable goal for both researchers and commercial companies. However, among reports of several methods to genetically transform these flowers, most are difficult to upscale for the practical introduction of novel traits, let alone reproduce. We have developed a unique and efficient transformation procedure resulting in the regeneration of non-chimeric transgenic carnation plants of several cultivars. The procedure is based on (i) combining microprojectile bombardment and *Agrobacterium*-mediated transformation, and (ii) two selection cycles. Wounding by microprojectile bombardment was found to markedly improve transient GUS expression in stem segments when performed prior to inoculation with *Agrobacterium*. Following cocultivation under a light regime that improved transient GUS expression in several standard and spray cultivars, a two-step selection cycle was employed for the recovery of non-chimeric transgenic shoots. In the first selection cycle, putatively transgenic plants were regenerated and selected from inoculated stem segments. These regenerants, some of which exhibited a pattern of chimeric GUS expression, were subjected to a second selection cycle, in which their leaves served as a source for the regeneration and selection of non-chimeric transformed shoots. With this procedure, an average 20 transgenic cv. White Sim plants, 8 transgenic cv. Desio plants, and 15 transgenic cv. Eilat plants, originating from 100 stem segments, were recovered, as confirmed by molecular and histochemical analyses. Using this protocol and various genes of interest, transgenic plants with dramatically altered plant morphology and flower color were generated.

Interactions Between Plant Auxin And Antibiotics And The Stability Of Plant Auxin On Plant Transformation. Jhy-Jhu Lin Agricultural Biotechnology R&D, Life Technologies Inc. (GIBCO BRL), Rockville, MD 20850

The toxic effects of kanamycin (Km) on plant tissues were attenuated when carbenicilin was added together with Km. The attenuation of Km toxicity was attributed to the contribution of the auxin activity of carbenicillin. Substitution with different concentrations of NAA for carbenicillin resulted in attenuation of Km toxicity in potato and soybean stems. Qualitative and quantitative analysis of plant auxins such as CPA, NAA, 2,4-D, MeOIAA, 5-ClIAA in MS medium, which is reconstituted from 50X concentrated liquid medium, was achieved by Capillary electrophoresis (CE). Only a 30% loss of IAA was observed after 3 weeks incubation at room temperature under a cycle of 16h light and 8 h dark. It was determined that 50% of IAA was degraded after 7 days incubation under continuous light exposure. Accelerated degradation (50%) of IAA or NAA was observed when the tobacco leaf discs were involved after 1.5 weeks incubation under a cycle of 16h light and 8 h dark. Therefore, by adjusting the levels of plant auxins in MS medium, the transformation efficiency of *Arabidopsis thaliana* was significantly improved from 20% to >90% using *Agrobacterium*-mediated transformation. These results indicate that optimization of plant transformation should take into consideration the interactions between antibiotics and plant growth regulators as well as the stability of the plant auxin in the culture medium.

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Abstract:
The temporal and spatial control of the transition from vegetative to parasitic mode is critical to any parasite, but essential to the sessile parasitic plants. This transition in Striga spp. has been proposed to be controled simply by an exuded oxidase which converts host cell surface phenols into the developmental signals mediating this transition. PoxA and PoxB were identified as the only apoplastic phenol oxidases from S. asiatica seedlings, and the genes have been cloned and sequenced. The catalytic activity of PoxA and PoxB explains the ability of the >60 known molecules which induce parasite development by mediating their conversion to a small subset of quinone xenognosins. However analysis of the reaction requirements and comparisons to host enzymes leads us to propose new model where constitutive production of activated oxygen species (AOS) mediates host recognition. This strategy allows a parasite to exploit abundant host enzymes for the production of diffusible xenognostic signals by converting a standard defense into an offense. The evolution of this as a general strategy and the signal transduction events involved in the activation will be discussed.

DIFFERENTIALLY EXPRESSED GENES INDUCED IN RESISTANT SOYBEAN PLANTS IN RESPONSE TO SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, INVASION

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinoe is a devastating pest of the soybean, *Glycine max* L. Merr, causing chlorosis, root necrosis, and suppressing shoot growth. We used differential display to identify genes expressed in a resistant soybean cultivar (Peking) compared to susceptible (Kent) and uninoculated plants. Gene expression was monitored 6, 12, 24, 48, 96 and 144 hours after inoculation with SCN. We identified and cloned over 50 unique DNA fragments by differential display, some which correspond to genes specifically induced in leaves or roots of resistant inoculated plants. The DNA sequences were compared against nucleotide and protein databases to identify putative gene function. We identified genes induced at an early stage of the infection process, such as transcription factors, nucleotide binding proteins, protein kinases and leucine-rich repeats. These genes are induced during nematode penetration and migration through the root. At a later stage, genes involved in general defense response were induced, coinciding with degeneration of the nematode feeding site. cDNA arrays are being tested to determine the response of other soybean genes to SCN invasion.

Transformation of carrot and tomato with a carrot heat shock protein gene, Hsp17.7, driven by the CaMV 35S promoter, enhances thermotolerance in cell lines and/or plants.

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The heat shock response of higher plants is distinct from that of animals in the abundanct production of a diverse set of low molecular weight heat shock proteins (lmw Hsps). It has been thought for some time that this low molecular weight class of Hsps might have a special significance in the plant heat shock response. We have manipulated the expression of a carrot gene encoding the Class I lmw Hsp, Hsp 17.7, by linking the coding sequence of the gene to the CaMv 35S promoter. Transformed cells and regenerated transgenic carrot plants, as well as transformed tomato plants, containing this construct (denoted CaS) were tested for themotolerance. Transformed carrot cultured cells were significantly more thermotolerant than vector control lines, as assayed for cell growth after exposure to a heat shock treatment. Moreover, second generation regenerated transgenic carrot plants, from multiple lines, were also significantly more thermotolerant than vector control transformed plants, as assayed by electrolyte leakage measurements. There were no other obvious differences in carrot cell culture growth and regeneration capability between the CaS cells and vector control lines. In addition, there were no observable differences in growth, morphology, time to flowering or seed set between CaS carrot plants and vector control plants. The two independent lines of transformed tomato plants we have assayed, so far, also exhibit increased thermotolerance in two types of assays, electrolyte leakage and greening of etiolated cotyledons. There were also no significant differences in growth characteristics between CaS tomato plants and vector control plants.
Preliminary molecular analysis of the CaS carrot cells suggests a low level of Hsp 17.7 mRNA accumulation prior to heat shock and a rapid accumulation of heat shock mRNA after exposure to elevated temperatures. Molecular analysis of the transgenic cells at the protein level will be discussed. Funded in part by a grant from NSF-IBN-9604607.

Development of a Novel Approach for the *In situ* Analysis of Transcription Factor-DNA Complexes

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A novel method for studying *in vivo* interactions between transcription factors and their promoter-DNA binding sites is being developed to elucidate the contribution of these factors to promoter activity. This method will specifically be utilized to evaluate the binding activity of the basic leucine-zipper (bZIP) transcription factor TGA1a to the *as*-1 promoter element in response to various cellular and environmental signals in plants. Further, this method will allow for direct screening of *as*-1 in plant genes that are regulated by this element.

This method applies the ability of formaldehyde to rapidly but reversibly form covalent cross-linkages between proteins and their promoter-DNA binding sites. Following enzymatic digestion of the plant cell wall, fixed plant cell chromatin is extracted and purified on a discontinuous Percoll gradient layered on a sucrose cushion. The chromatin is then cleaved into manageable fragments by DNase I to facilitate immuno-enrichment for specific transcription factor-promoter DNA complexes. Following thermal reversal of the formaldehyde-induced cross-links, the target transcription factor will be identified using Western blot analysis, with identification of the specific promoter-DNA sequence by PCR.

This method is likely to be generally useful to elucidate the pathways of gene expression via transcription factor interaction with promoter DNA.

Auxin-induced stress potentiates *trans*-activation by a conserved plant basic/leucine-zipper factor

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In plants, the promoter element "activation sequence-*1*" (*as-1*) confers transcription in response to diverse hormone and chemical-stress cues. One or more plant basic/leucine-zipper (bZIP) proteins, termed "TGA factors", are thought to mediate these transcriptional responses. We have shown here that a highly-conserved TGA factor of tobacco, TGA1a, can selectively activate transcription in response to micromolar concentrations of auxin hormones or their analogs. This induction is chemically-specific, as a range of other compounds tested at similar concentrations had little or no effect. Collectively, studies here revealed that the primary mode of action of auxin is to augment the *trans*-activation potential of TGA1a, largely through the carboxy-terminal (CT) domain of this factor. Residues in the amino-terminal (NT) domain of TGA1a, by gain-of-function assays, constitutively activate transcription and help to maximize the auxin-response. TGA1a and endogenous TGA factors are activated by auxin only at concentrations that inhibited cell growth, thus indicating that auxin acts here through a nonmitogenic pathway involving chemical-stress. This view is further supported by the observation that a biologically-inactive analog of auxin can similarly induce these responses.

A molecular genetic screen for factors that regulate *as-1*-dependent transcription in plants

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The *cis*-element "activation sequence-1" (*as-1*) confers gene expression in response to auxin hormones in plants. To complement our ongoing studies of how auxins modulate the *trans*-activation potential of specific *as-1*-binding factors, we will employ a molecular genetic screen to identify other plant proteins (e.g., nuclear cofactors and signal-transduction intermediates) that regulate transcription through *as-1*. By agrobacterium-mediated transformation we stably induced the *uidA* (GUS) and NPTII (Kanamycin-resistance) genes into *Arabidopsis thaliana* (Columbia ecotype). The *uidA* transgene is linked to minimal promoter sequences and a single upstream *as-1* element, which together confer root-specific and auxin-responsive expression of GUS in the transformants. Seeds from homozygous plants will be chemically-mutagenized, then germinated and collected in batches. Each batch will then be screened by plating on filter paper and incubating such that parallel root growth occurs. In the first screen, each batch of seedlings will be visually evaluated for as-1 dependent expression of the *uidA* reporter gene with a destructive GUS histochemical assay that stains cells blue. This screen, performed without prior chemical stress, will indicate gain of function mutants in each batch, as *as-1* normally down-regulates transcription. The second screen will indicate loss of function mutants that do not respond to chemical stress cues by up-regulating auxin-responsive transcription as is typical of *as-1*. This second screen includes an auxin treatment before the GUS assay and evaluation. Putative mutants will then be re-screened as above to eliminate false positives. Analysis of each mutant by CAP's (co-dominant cleaved amplified polymorphism's) will determine whether the mutation co-segregates with the GUS reporter gene. Genetic complementation tests between each mutant line will reveal whether different genes or alleles are involved. Finally, we will measure by RT-PCR the expression of several native genes to determine whether the mutation in each case selectively affects transcription through *as-1*. Mutants obtained from these genetic screens will be useful tools for revealing how constitutive and auxin-responsive transcription are regulated in plants.

MOLECULAR CLONING AND CHARACTERIZATION OF A WOUND-INDUCIBLE CYTOCHROME P450 FROM *NICOTIANA PLUMBAGINIFOLIA* TRANSFORMED WITH THE BACTERIAL ISOPENTENYL TRANSFERASE GENE.

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Plant cytochrome P450 monooxygenases are heme-containing enzymes that participate in the synthesis of a wide variety of secondary products, some of which are shown to inhibit insects, pathogens and animal herbivores. Using reverse transcription-polymerase chain reaction (RT-PCR) of poly(A)$^+$ RNA from *Nicotiana plumbaginifolia* containing the potato inhibitor wound-inducible promoter-isopentenyl transferase gene construct (PI-II-*ipt*), two full length clones of P450, designated as pNpl1 and pNpl2, were isolated and sequenced. pNpl1 has an open reading frame of 1524 nucleotides corresponding to 508 amino acids and its deduced amino acid sequence has 44% identity to *Catharanthus roseus* P450 (CYP72). pNpl2 is similar to pNpl1 except for 81 nucleotides deletion and an internal stop codon, and so possibly represents a pseudogene. When *in vitro* transcribed and translated, two ^{35}S-methionine labeled polypeptides with molecular masses of 56 and 34 kDa were synthesized corresponding to the products of pNpl1 and pNpl2, respectively. The complete coding region of pNpl1 was amplified by PCR and used to estimate the copy number of P450 genes and to study the expression of P450 in PI-II-*ipt*-transformed and normal *N. plumbaginifolia*.. Southern blot hybridization of genomic DNA indicated that P450 exists as multiple copies of the same gene. The expression of P450 in the leaves is regulated by light and darkness. Northern blot analysis revealed that transcript accumulation was maximum during the day but was lowest at night. When infested with tomato hornworm (*Manduca sexta*) larvae or mechanically wounded, this rhythm was disrupted resulting in an elevated level of expression at night in the wounded leaves. The level of induction was 4.5- to 6-fold higher in PI-II-*ipt*-transformed leaves after 6 to 12 h of mechanical wounding in comparison to a 2- to 3.5 fold induction from wounded but untransformed leaves. The response to feeding insect larvae was systemic and was detected maximally in the leaf immediately above the damaged leaf. P450 transcripts were not detected in flower buds, flowers and seed pods. Polyclonal antibodies were raised against a cocktail of three synthetic peptides whose sequences corresponded to internal regions of the deduced P450 protein exhibiting high antigenic indices. Preliminary Western blot analysis of cell-free extracts indicated the presence of 58.8 kDa P450 proteins in tobacco, periwinkle, sugarbeet and soybean leaves. The modulation of P450 gene expression by cytokinins and the possible role of P450 in plant defense responses are discussed.

ISOLATION OF A CYSTEINE PROTEINASE cDNA FROM THE ALFALFA WEEVIL AND ANALYSIS OF ITS MIDGUT PROTEINASES.

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Insects rely on a variety of midgut proteinases to catalyze the release of free amino acids from dietary protein and thereby provide nutrients essential for normal growth and development. A potential for insect control has been demonstrated in laboratory studies involving the expression of proteinase inhibitor (PI) genes in transgenic plants. However, insects have revealed an ability to compensate for lost proteinolytic activity by enhancing production of proteinases insensitive to the introduced PI. Thus, there is a need to characterize the individual proteolytic enzymes within an insect in order to pursue a directed control strategy in which each proteolytic activity is specifically targeted for inhibition. We are conducting both biochemical and molecular cloning experiments to elucidate the digestive proteinases of *Hypera postica*. Gelatin-containing SDS-PAGE of weevil midgut extracts has revealed one major and several minor size-classes of proteolytic activity. The large majority (70-80%) of proteolytic activity appears to result from cysteine proteinases in the midgut extract, as revealed by inhibition of the enzymatic activity with class-specific protease inhibitors. Of interest from the standpoint of pest control, the recombinant rice inhibitors OCI and OCII were similarly effective at inhibiting proteolytic activity as the potent, irreversible cysteine proteinase inhibitor, E-64. One cysteine proteinase clone has been identified in a random sampling of 10 lambda clones from an *H. postica* midgut-specific cDNA library. DNA sequencing of the insert has revealed a full-length cDNA (*hcp1*) encoding a predicted protein (HCP1) of 324 amino acids. This putative digestive enzyme is highly similar to cathepsin L-type cysteine proteases, and is predicted to play an important role in the assimilation of dietary protein in the alfalfa weevil.

Two-hybrid Interactions of the *Arabidopsis* CTR1 Raf-like Kinase with 14-3-3 Proteins

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The *Arabidopsis* CTR1 gene product, which has sequence similarity to the Raf family of serine/threonine protein kinases, acts downstream of the putative ethylene receptors in the ethylene signal transduction pathway. We are interested in understanding the signaling events that lead from the perception of ethylene to the activation of CTR1. Toward this end, we are using the yeast two-hybrid assay to identify proteins that interact with the putative regulatory domain (N-terminal domain) of CTR1 and/or the ethylene receptors. Using an N-terminal portion of CTR1 as a bait, we screened an etiolated seedling library of *Arabidopsis*, and analyzed 28 of the strongest positive clones. Based on sequence analysis, 21 of these clones encode 14-3-3 proteins, which represent five of the ten known 14-3-3 isoforms in *Arabidopsis.* All of the clones contain the C-terminus and are missing the N-terminus to different extents. None of the clones display interaction with the kinase domain of CTR1 nor with human lamin (a negative control). Interestingly, we found that the ETR1 ethylene receptor can interact in the two-hybrid assay with one of the 14-3-3 isoforms. In mammals, several 14-3-3 isoforms can interact physically with Raf, and seem to play a role in Raf activation. These findings suggest the possibility that 14-3-3 proteins have a role in the ethylene signal transduction pathway in *Arabidopsis*. This work is supported by USDA/NRICGP grant no. 95-37304-2218.

Analysis of Peroxisomal Targeting of the Soybean Glyoxysomal Aspartate Aminotransferase Expressed in Yeast.

Michael S. Paluch and Gregory J. Wadsworth, Department of Biology, Buffalo State College, 1300 Elmwood Ave., Buffalo, NY 14222.

Aspartate aminotransferase (AAT) catalyzes the transfer of the amino group of aspartate to 2-oxoglutarate to form oxaloacetate and glutamate. Five isozymes of AAT have been distinguished in soybean, and are designated AAT-1 to AAT-5. The AAT-1 isozyme is localized in the glyoxysomes of soybean. Sequence analysis of the AAT-1 gene has identified a non consensus glyoxysomal PTS-2 type targeting sequence in the N-terminal of the protein [1]. To determine if this is a functional targeting sequence, the AAT-1 gene was expressed in yeast. Two expression constructs were produced, the first construct expressing a full length AAT-1 containing the PTS-2 targeting sequence, and a second construct expressing a truncated AAT-1 lacking the PTS-2 targeting sequence. Differences in growth rates were observed for cells containing the two expression constructs. When grown on media requiring peroxisomal function, cells expressing the full length AAT-1 grew significantly slower than cells expressing the truncated AAT-1. This suggests that overexpression of the PTS-2 of AAT-1 may interfere with peroxisome function. Subcellular location of soybean AAT in yeast was assayed by sucrose gradient centrifugation. In cells with the truncated transcript, all of the AAT activity remained soluble. Some of the AAT in cells with the full length expression vector co-fractionated with peroxisomal marker enzymes (catalase). However, most of the AAT activity remained soluble and did not co-fractionate with any organelle. This suggests the N-terminal domain of AAT-1 plays some role in peroxisomal targeting.

1. Gebhardt J.S., Wadsworth G.W., and Mathews B.F. (1998) Characterization of a single soybean cDNA encoding cytosolic and glyoxysomal isozymes of aspartate aminotransferase. Plant Molecular Biology 37: 99-108.

Soybean Aspartate Aminotransferase-1 Gene; Evidence for Use of Two Distinct Start Codons using Yeast Expression System.

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The Aspartate Aminotransferase-1 (AAT-1) gene of soybean contains two putative start codons. We have proposed that the first start codon intiates synthesis of the glyoxysomal form of the isozyme and that the second start codon intiates synthesis of the cytosolic form of the isozyme. To investigate whether both start codons are functional and produce aspartate aminotransferase we cloned the AAT-1 gene into an expression vector for S. cerveseae. Two constructs were produced; a full length gene that contained both start codons (ATG1 and ATG2) and a second truncated gene that contained only the second start codon (ATG2). Both constructs produced functional AAT enzymes as assayed spectrophotometrically or by native gel electrophoresis. Western blot analysis was used to determine the size of the AAT polypeptide produced by the expression vector. The truncated gene (with only ATG2) produced a single polypeptide Mr 40Kd. The full length gene (both ATG1 and ATG2) produced a 40 Kd and a 43 Kd polypeptide. The presence of these two polypeptide bands suggests that both start codons of soybean AAT are utilized in yeast.

THE GENOMES OF RAPID-CYCLING BRASSICAS ARE NO SMALLER THAN THOSE OF THEIR WILD-TYPE PROGENITORS:, Jennifer Hansen, Lori Lasco, Jessica A. Chinault, John J. Kerrigan, Mary Fields and Robert Dawley; Department of Biology, Ursinus College, Collegeville, PA 19426-1000.

Small genomes have been associated in eukaryotes with reduced cell volume, increased metabolic and developmental rate, a shortening of the cell cycle, and a reduction in minimum generation time. Arabidopsis, for example, has the smallest genome measured among angiosperms and a remarkably short generation time. We hypothesized that rapid-cycling Brassicas, derived from wild strains of Brassica by artificial selection for short generation time, would have smaller genomes than those of their wild-type progenitors. But they do not. We used flow cytometry to measure diploid genome sizes (in pg DNA) of rapid-cycling strains of the following species: Brassica carinata, B. juncea, B. napus, B. nigra, B. oleracea, B. rapa, and Raphinus sativa, and found that genome sizes of the rapid-cyclers do not differ from values reported in the literature for their wild-type progenitors. Perhaps the genomes of these Brassica species are so small (1.0-2.6 pg DNA) that reduction of generation time via elimination of non-genic DNA is not a route that selection can take. *Supported by grants from HHMI, NSF (ILI Grant #9050888), and Ursinus College (Van Sant Fund).*

TRANSIENT EXPRESSION OF *RPM1* AND *RPS2* WITH THEIR COGNATE AVIRULENCE GENES IN *ARABIDOPSIS* PROTOPLASTS TO STUDY GENE-FOR-GENE DISEASE RESISTANCE.

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The *Arabidopsis thaliana* resistance genes *RPS2* and *RPM1* confer gene-for-gene disease resistance to the bacterial phytopathogen *Pseudomonas syringae* carrying the avirulence genes *avrRpt2* and *avrB*, respectively. Both *RPS2* and *RPM1* belong to the nucleotide binding site (NBS)-leucine rich repeats (LRR) class of resistance genes which contains members conferring resistance to all major pathogens of plants. Using a quantitative transient expression assay in *Arabidopsis* leaf mesophyll protoplasts, we show that expression of *RPS2* or *RPM1* with its cognate avirulence gene can elicit a specific resistance response. In this assay, the avirulence and resistance genes to be tested are introduced into protoplasts along with the reporter gene for luciferase (LUC) using polyethylene glycol (PEG) mediated transformation. An incompatible avirulence-resistance gene combination results in rapid cell death and limits the accumulation of the luciferase reporter. These observations demonstrate that protoplasts are capable of specifically recognizing avirulence gene-based signals and that some of the specific resistance response is cell-autonomous. Using this simplified system we are further characterizing the interaction between avirulence and resistance gene products with *in vivo* labeling and immunoprecipitation. The cell-autonomous signal transduction pathway activated in protoplasts by the gene-for-gene interaction is also being explored with the addition of specific signal transduction inhibitors.

NOVEL APPROACH TO DETECT PROTEIN TRANSFER FROM A BACTERIAL PATHOGEN TO ITS PLANT HOST

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A plant can recognize a pathogen and be resistant against it when the pathogen carries an appropriate avirulence (avr) gene. Some indirect evidence strongly suggests that in the case of bacterial pathogens the avr gene product is transported via the Type III secretion system from the pathogen into the plant cell where the molecular recognition takes place. In *Pseudomonas syringae*, a cluster of genes known as *hrp* (hypersensitive response and pathogenicity) encode the components of the Type III secretion system. To obtain a direct demonstration of the putative protein transport, we have been using fusion proteins composed of an Avr protein and the eukaryotic transcription activator GAL4-VP16. GAL4-VP16 (GV) can activate transcription from the promoter with its cognate binding site (UAS). *Arabidopsis thaliana* plants transformed with a luciferase reporter gene linked to the UAS containing promoter are infected with bacterial strains that carry the fusion genes. If the protein transport indeed occurs, it should be detected as an increase in the LUC expression. We have been using *P. syringae* and *E. coli* expressing *hrp* genes for the transport assay. The fusions were also characterized by direct transient expression in plants for their avirulence and transcription activation functions. In this way, we could test if fusions have any problems other than the protein transport process when negative results for protein transport are obtained. We made fusion constructs using four *P. syringae* avr genes, *avrB*, *avrRpt2*, *avrRpm1*, and *avrPto*. C-terminal fusions of GV to all Avr proteins except AvrRpt2 failed to activate transcription of the reporter when directly expressed in the plant, and transport of AvrRpt2GV was not detected. While N-terminal fusions of GV to AvrB and AvrPto function as transcription activators, their transport was not detected. Three insertions of GV into AvrB were constructed, none of which could activate transcription when expressed in plants. Currently, insertions of GV into AvrRpt2 are being tested.

The Effect Of Tissue Culture Conditions On Auxin Stability In Culture Media.

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Abstract

Auxin stability in culture media is critical for understanding the mechanism of plant regeneration. Degradation of auxin in MS medium is related to pH, salt concentration, light exposure, medium sterilization and involvement of plant tissues or calli. Inconsistent results have been reported on auxin stability.

In this study, we use capillary electrophoresis (CE) to analyze IAA in the MS medium which is reconstituted from 50X concentrated liquid MS medium. Both qualitative and quantitative analyses of various auxins such as CPA, IAA, NAA, 2,4-D, MeoIAA, 5-ClIAA were carried out. In a Magenta container, 50% IAA degradation was observed after 7 days at 25°C for solutions containing 2mg/l of IAA in the MS medium under continuous light. In contrast, only 30% IAA loss was found after 3 weeks for the same solutions under a cycle of 16h light and 8h dark. The complete IAA degradation was not observed until 8 weeks. The auxin degradation is much more rapid when tobacco leaf discs are involved. About 50% IAA or NAA was lost after 1.5 weeks under the same cycle of 16h light and 8 h dark. In addition, after examining various plastic or glass containers for light absorbency, a wide range of light absorbency (from 277nm to 355nm) was observed. Therefore, our results demonstrate that (1) the rapid degradation of auxins in tissue culture is primarily derived from the presence of plant tissues; (2) the slower IAA degradation than literature reports may be due to the difference of medium formulation, medium preparation, tissue culture containers, and incubation condition.

Plant Nucleic Acid Isolation Using Guanidine based Reagents

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ABSTRACT

Rigid cell wall, polysaccharides, and polyphenolic compounds complicate the extraction of high quality DNA and RNA from plant tissues. In this report we describe two new chemical reagents that not only simplify nucleic acid isolation processes but also generate high yield of genomic DNA and total RNA from plant tissues. In plant genomic DNA isolation a guanidine-detergent lysing solution, Plant DNAzol Reagent, is specifically formulated for the isolation of genomic DNA from plants. Plant DNAzol Reagent allows the selective precipitation of DNA from the lysate and hydrolyzes contaminated RNA. Typically 2~10 ug of genomic DNA is recovered from 0.1 g of plants like tobacco, rice, potato and maze or different plant tissues like leave, root and stem. In addition, it only requires about half the time to perform comparing with the conventional CTAB method and does not require an additional treatment to eliminate RNA. In plant RNA isolation, TRIzol Reagent, a mono-phasic solution of phenol and guanidine isothiocyanate is used to isolate 0.3~1.6 mg of total RNA from 1 g of leaf tissues in a single isolation step. The quality of nucleic acid isolated from these reagents was demonstrated by different applications such as Southern-blot hybridization, Northern-blot hybridization, PCR amplification, AFLP marker detection and cDNA synthesis successfully.

Comparing Digital Images To Conventional Photographs

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Digital cameras, unlike conventional cameras, instantaneously digitalize images, increasing the speed and flexibility by which data can be electronically analyzed and distributed. Scientific data documented by a conventional camera and a digital camera was compared using pictures and images of a field plant, tissue culture calli, agrose gel electrophoresis and chemiluminescent detected AFLP will be discussed. Color images of potato regeneration showing calli and a fully regenerated plant were compared with few noticeable differences. The scanned images of an electrophoresed gel and a non-rad AFLP show no significant differences from the digital camera version. Furthermore, quantitative analyses of the gels with *Kodak Digital Science ID Image Analysis Software* yield similar results between the conventional picture and Digital image. These results demonstrate that the digital camera is a viable alternative to a conventional camera. In addition, the digital camera is fast and allows for convenient computer integrated for data preparation, analysis and distribution.

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